CLAIMS

What is claimed is:

1. A method for detecting in real time the amount of a target nucleic acid molecule in a sample, wherein the melting of the target nucleic acid molecule starts at a temperature T_{MS} and completes at a temperature T_{ME}, the method comprising:

A. Establishing a standard curve by:

i) PCR-amplifying, in the presence of a suitable fluorescent dye, the target nucleic acid molecule, with a known starting concentration (C) through cycles of denaturing, annealing, and chain extension, wherein the fluorescence is increased when the dye is combined with a double-stranded nucleic acid molecule, wherein the chain extension occurs at a chain extension temperature T_E;

ii) measuring the fluorescence (F) during each amplification cycle at the temperature immediately before the temperature starts to increase from T_E (F_E at T_E), at any temperature point (T_B) in between T_E and T_{MS} (F_B at T_B), at T_{MS} (F_{MS} at T_{MS})and at T_{ME} (F_{ME} at T_{ME});

iii) calculating a baseline slope (S_B), defined by negative (F_B minus F_E), divided by (T_B minus T_E), and an amplicon melting phase slope (S_M), defined by negative (F_{ME} minus F_{MS}) divided by (T_{ME} minus T_{MS});

iv) recording the number of PCR cycles (N) required for the quantity (S_M minus S_B) to first become greater than zero;

v) repeating steps i) through v) for a suitable range of concentrations of interest; and

vi) plotting C against C_T to obtain a standard curve for the target nucleic acid sequence; and

B. Repeating steps (A) (i) through (A)(v) for a sample containing an unknown concentration of the target nucleic acid molecule, to obtain an C_T value for the sample, and determining the target nucleic acid molecule concentration via the standard curve.

2. The method of Claim 1 wherein the sample contains a first target nucleic acid molecule and a second target nucleic acid molecule, wherein the melting of the first target nucleic acid molecule starts at a temperature T_{MS1} and completes at a temperature T_{ME1}, the melting of the second target nucleic acid molecule starts at a temperature T_{MS2} and completes at a temperature T_{ME2}, and wherein T_{MS2} is greater than T_{ME1}, the method comprising:

A. Establishing a standard curve for each of the target nucleic acid molecule by:

i) simultaneously PCR-amplifying, in the presence of a suitable fluorescent dye, the target nucleic acid molecules with a known starting concentration (C_1 and C_2) through cycles of denaturing, annealing, and chain extension, wherein the fluorescence is increased when the dye is combined with a

double-stranded nucleic acid molecule, wherein the chain extension occurs at a chain extension temperature T_E;

ii) measuring the fluorescence (F) during each amplification cycle at the temperature immediately before the temperature starts to increase from T_E (F_E at T_E), at any temperature point (T_{B1}) in between T_E and T_{MS1} (F_{B1} at T_{B1}), at T_{MS1} (F_{MS1} at T_{MS1}), at T_{ME1} (F_{ME1} at T_{ME1}), at any time point (T_{B2}) in between T_{ME1} and T_{MS2} (F_{B2} at T_{B2}), at T_{MS2} (F_{MS2} at T_{MS2}), at T_{ME2} (F_{ME2} at T_{ME2});

iii) calculating a baseline slope for the first target molecule (S_{B1}), defined by negative (F_{B1} minus F_E), divided by (T_{B1} minus T_E), and a first amplicon melting phase slope for the first molecule (S_{M1}), defined by negative (F_{ME1} minus F_{MS1}) divided by (T_{ME1} minus T_{MS1}); and calculating a baseline slope for the second target molecule (S_{B2}), defined by negative (F_{B2} minus F_{ME1}), divided by (T_{B2} minus T_{ME1}), and a melting phase slope for the first molecule (S_{M2}), defined by negative (F_{ME2} minus F_{MS2}) divided by (T_{ME2} minus T_{MS2});

iv) recording the number of PCR cycles (N_1) required for the quantity (S_{M1} minus S_{B1}) to first become greater than zero; and recording the number of PCR cycles (N_2) required for the quantity (S_{M2} minus S_{B2}) to first become greater than zero;

v) repeating steps i) through v) for a suitable range of concentrations of interest for each of the two target molecules; and

vi) plotting C_1 against N_1 to obtain a standard curve for the first target molecule; and plotting C_2 against N_2 to obtain a standard curve for the second target molecule; and

B. Repeating steps (A) (i) through (A)(v) for a sample containing an unknown concentration of the first and second target nucleic acid molecules, to obtain an N_1 value and an N_2 value for the sample, and determining the target nucleic acid molecule concentrations via the standard curve.

3. The method of Claim 1 wherein the sample contains a first target nucleic acid molecule, a second target nucleic acid molecule and a third target nucleic acid molecule, wherein the melting of the first target nucleic acid molecule starts at a temperature T_{MS1} and completes at a temperature T_{ME1}, the melting of the second target nucleic acid molecule starts at a temperature T_{MS2} and completes at a temperature T_{ME2}, the melting of the third target nucleic acid molecule starts at a temperature T_{MS3} and completes at a temperature T_{ME3}, and wherein T_{MS3} is greater than T_{ME2}, the method comprising:

A. Establishing a standard curve for each of the target nucleic acid molecule according to the method of Claim 1;

B. Simultaneously PCR amplifying a sample containing an unknown concentration of the target nucleic acid molecules, to obtain an N_1, N_2 and N_3 value for the sample, and determining the target nucleic acid molecule concentrations via the standard curve.

4. The method of Claim 1 wherein the sample contains n target nucleic acid molecules, wherein n is an integer greater than three, wherein the melting of the first target nucleic acid molecules starts at a temperature T_{MS1} and completes at a temperature T_{ME1}, the melting of the second target nucleic acid molecule starts at a temperature T_{MS2} and completes at a temperature T_{ME2}, the melting of the $(n-1)^{th}$ target nucleic acid molecule starts at a temperature $T_{MS(n-1)}$ and completes at a temperature $T_{ME(n-1)}$, the melting of the n^{th} target nucleic acid molecule starts at a temperature T_{MSn} and completes at a temperature T_{MEn}, and wherein T_{MSn} is greater than $T_{ME(n-1)}$, the method comprising:

A. Establishing a standard curve for each of the target nucleic acid molecule according to the method of Claim 1;

B. Simultaneously PCR amplifying a sample containing an unknown concentration of the target nucleic acid molecules, to obtain an N_1, N_2 . . . and N_n value for the sample, and determining the target nucleic acid molecule concentrations via the standard curve.

5. The method of Claim 2, wherein the first and the second target nucleic acid molecules reside on the same genome of an organisms, and wherein the copy number per genome for the first target nucleic acid molecule is known, whereby the copy number per genome for the second target nucleic acid molecule is determined.

6. The method of claim 1, wherein the target nucleic acid molecule is from a pathogenic organisms.

7. The method of Claim 2, wherein the first target nucleic acid is an invertase gene, an aldolase gene or a lectin gene, and wherein the second target nucleic acid is selected from the group consisting of the 35S CaMV promoter, a Cry9C gene, an GA21 gene, an EPSPS (5-enolpyruvylshikimate-3-phosphate synthase gene, a PEPC promoter; an hsp70 promoter of Cry1A(b) gene, a Cry1A(b) gene; an NOS gene, and the actin promoter gene.

8. The method of Claim 1 wherein the target nucleic acid molecule is selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:9, and SEQ ID NO:10.

9. The method of claim 1, wherein the target nucleic acid molecule is a nucleic acid fragment is part of a transgene contained in a genetically modified organism.

The method of claim 9 wherein the target nucleic molecule comprises a promoter for the transgene.

10. The method of claim 10 wherein the promoter is the 35S promoter of Cauliflower Mosaic Virus.